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Bioinformatics analysis and prediction for structure and function of nitric oxide synthase and similar proteins from *Plasmodium berghei*Zhigang Fan<sup>1\*</sup>, Gang Lv<sup>1</sup>, Lingmin Zhang<sup>2</sup>, Xiufeng Gan<sup>1</sup>, Qiang Wu<sup>1</sup>, Saifeng Zhong<sup>1</sup>, Guogang Yan<sup>3</sup>, Guifen Lin<sup>1</sup><sup>1</sup>School of Tropical and Laboratory Medicine, Hainan Medical University, Haikou 571101, P.R. China<sup>2</sup>Department of Parasitology, Medical School, Jinan University, Guangzhou 510632, P.R. China<sup>3</sup>School of Nursing, Hainan Medical University, Haikou 571101, P.R. China

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## ABSTRACT

**Objective:** To search and analyze nitric oxide synthase (NOS) and similar proteins from *Plasmodium berghei* (Pb). **Methods:** The structure and function of nitric oxide synthase and similar proteins from *Plasmodium berghei* were analyzed and predicted by bioinformatics. **Results:** PbNOS were not available, but nicotinamide adenine dinucleotide 2'-phosphate reduced tetrasodium (NADPH)–cytochrome p450 reductase (CPR) were gained. PbCPR was in the nucleus of *Plasmodium berghei*, while 134aa–229aa domain was localized in nucleolar organizer. The amino acids sequence of PbCPR had the closest genetic relationship with *Plasmodium vivax* showing a 73% homology. The tertiary structure of PbCPR displayed the forcep-shape with wings, but no wings existed in the tertiary structure of its' host, *Mus musculus* (Mm). 137aa–200aa, 201aa–218aa, 220aa–230aa, 232aa–248, 269aa–323aa, 478aa–501aa and 592aa–606aa domains of PbCPR showed no homology with MmCPRs', and all domains were exposed on the surface of the protein. **Conclusions:** NOS can't be found in *Plasmodium berghei* and other *Plasmodium* species. PbCPR may be a possible resistance site of antimalarial drug, and the targets of antimalarial drug and vaccine. It may be also one of the mechanisms of immune evasion. This study on *Plasmodium berghei* may be more suitable to *Plasmodium vivax*. And 137aa–200aa, 201aa–218aa, 220aa–230aa, 232aa–248, 269aa–323aa, 478aa–501aa and 592aa–606aa domains of PbCPR are more ideal targets of antimalarial drug and vaccine.

## 1. Introduction

Nitric oxide (NO) is an important gaseous signal molecule in biological tissue. Nitric oxide synthases (NOS) include neuronal nitric oxide synthase (nNOS or NOS1), inducible nitric oxide synthase (iNOS or NOS2) and endothelial nitric oxide synthase (eNOS or NOS3). Recently, NO has been found in or around the food vacuole in *Plasmodium falciparum* parasites [1], but no NOS is found in *Plasmodium falciparum*. Using the bioinformatics database and software, this article is to search NOS and similar proteins from *Plasmodium berghei* (Pb) and analyze their structure and function.

## 2. Materials and methods

The gene sequence, mRNA and amino acids (aa) of NOS from *Homo sapiens* (Hs) and *Mus musculus* (Mm) were obtained from NCBI database (<http://www.ncbi.nlm.nih.gov>), then the amino acids sequences of NOS and similar proteins from Pb were blasted. The physical–chemical properties were predicted bases on <http://www.expasy.org/tools/protparam.html>, and the molecule evolution was analyzed by ClustalX2. The sub-cellular localization of the protein, signal peptide, secondary structures, topological structure and 3D structure were predicted according to <http://psort.nibb.ac.jp/form2.html>, <http://www.cbs.dtu.dk/services/SignalP/>, <http://www.predictprotein.org/>, and [http://geno3d-pbil.ibcp.fr/cgi-bin/geno3d\\_automat.pl?page=GENO3D/geno3d\\_home.html](http://geno3d-pbil.ibcp.fr/cgi-bin/geno3d_automat.pl?page=GENO3D/geno3d_home.html), respectively.

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3.Results

3.1. PbNOS and similar proteins

The accession number of the gene, mRNA and aa of *HsNOS* and *MmNOS* were listed in Table 1.

No protein with similar mRNA or aa to *HsNOS* and *MmNOS* was found in *Pb* and other *Plasmodium* species, but nicotinamide adenine dinucleotide 2'-phosphate reduced tetrasodium (NADPH)NADPH-cytochrome p450 reductase

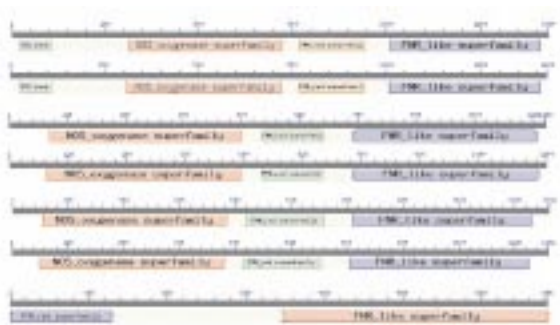
(CYPOR or CPR) of *Pb* was found in part similar to *HsNOS* and *MmNOS*. Gene ID of *PbCPR* was 3426618, and the accession number of mRNA and aa of *PbCPR* were XM\_672962.1 and XP\_678054.1. The amino acid sequence of *PbCPR* was really just like C-terminal amino acid sequence of *HsNOS* and *MmNOS*, which includes FNR-like superfamily and FMN-red superfamily. *PbCPR* 1aa-144aa was located in conserved domains of FMN-red superfamily, while 323aa-684aa in domains of FNR-like superfamily(Table 2 and Figure 1).

**Table 1**  
Accession number of the gene, mRNA and aa of *HsNOS* and *MmNOS*.

	NOS1		NOS2		NOS3	
	<i>Hs</i>	<i>Mm</i>	<i>Hs</i>	<i>Mm</i>	<i>Hs</i>	<i>Mm</i>
Gene	NG_011991.1	–	NG_011470.1	–	NG_011992.1	–
mRNA	NM_000620.2	NM_008712.2	NM_000625.4	NM_010927.3	NM_000603.4	NM_008713.4
Amino acids	NP_000611.1	NP_032738.1	NP_000616.3	NP_035057.1	NP_000594.2	NP_032739.3

**Table 2**  
Similarity of amino acid sequence of *Pb* CPR, *HsNOS* and *MmNOS*.

Sequence			Sequence			Sequence		
<i>Pb</i> CPR	1 – 136	349 – 655	<i>Pb</i> CPR	1 – 144	349 – 658	<i>Pb</i> CPR	1 – 136	349 – 655
<i>HsNOS1</i>	769 – 941	1092 – 1367	<i>HsNOS2</i>	548 – 686	821 – 1098	<i>HsNOS3</i>	529 – 704	852 – 1127
<i>Pb</i> CPR	1 – 142	349 – 638	<i>PbCPR</i>	1 – 144	342 – 680	<i>Pb</i> CPR	1 – 655	–
<i>MmNOS1</i>	764 – 942	1087 – 1362	<i>MmNOS2</i>	349 – 658	815 – 1092	<i>MmNOS3</i>	528 – 1126	–



**Figure 1.** Conserved domains of *HsNOS*, *MmNOS* and *PbCPR*.

3.2. Physical-chemical property of PbCPR

As a non secretory protein, *PbCPR* is in cell nuclei, 684 aa in length, ans 80237.7 Da in molecular weight. It is unstable and the pI is 8.70.

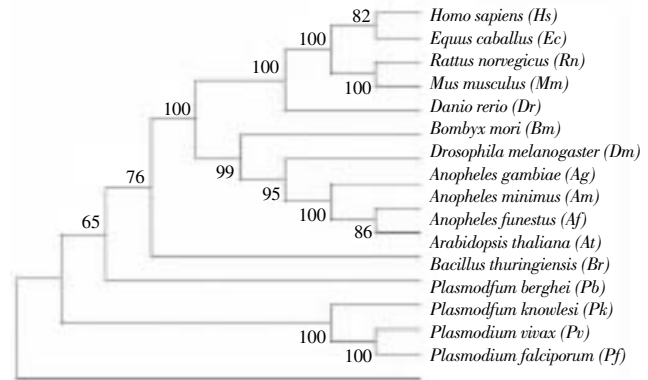
3.3. Molecular evolution of PbCPR

There were 397 highly conserved amino acids in *PbCPR* which were homologous to those of *Plasmodium vivax* CPR (*PvCPR*). The rates of consistency and similarity were 57% and 73%, respectively. While there were 102 highly conserved amino acid which were homologous to those of *Plasmodium falciparum* CPR (*PfCPR*). And the rates of consistency and similarity were 27% and 48% between *PbCPR* and *PfCPR*. *PbCPR* had close relationship with *PvCPR* but distant from *PfCPR*, *MmCPR* and *HsCPR*(Figure 2).

3.4. Structure of PbCPR

$\alpha$ -helics accounted for 31.7%,  $\beta$  strands for 14.5%, and loop for 53.9% in the secondary structure of *PbCPR*. 134aa-229aa was localized in nucleolar organizer regions (NORs) of *Plasmodium berghei*. A total of 50.58% amino acid were exposed on the surface of the protein. *PbCPR* had 5 N-glycosylation sites, 2 cAMP- and cGMP-dependent protein kinase phosphorylation sites, 14 protein kinase C phosphorylation sites, 6 casein kinase II phosphorylation sites, 3 tyrosine kinase phosphorylation sites, and 1 N-myristoylation site (Figure 3 and 4).

The tertiary structure of *PbCPR* showed forceps with wings. Conserved domaind of FMN-red superfamily and FNR-like superfamily formed a huge and deep fracture. 134aa-229aa was localized in upper left of the back of the protein. Most functional sites scattered on the surface of the protein(Figure 4).



**Figure 2.** Molecular evolution analysis of *PbCPR*.

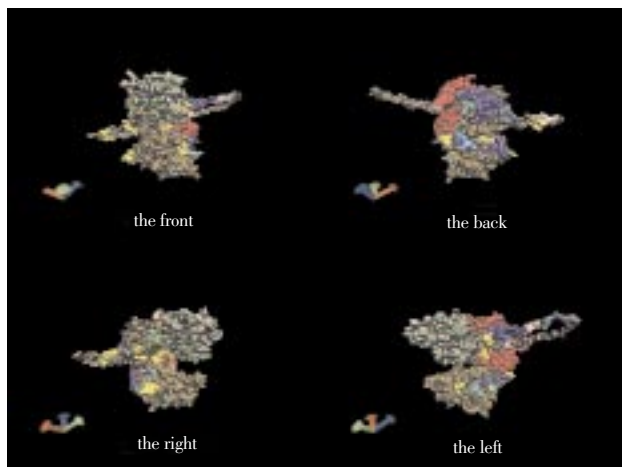
### 3.5. Comparison of *PbCPR* and *MmCPR*

*PbCPR* was in cell nuclei, while *MmCPR* was in cytoplasm. 89aa–233aa of *MmCPR* and 1aa–144aa of *PbCPR* lied in conserved domain of FMN–red superfamily with 49 homologous highly conserved amino acids. The rates of consistency and similarity were 31% and 54% between *PbCPR* and *PvCPR*. 343–677aa of *MmCPR* and 323aa–684aa of *PbCPR* lied in conservative structure domain of FNR–like superfamily with 103 homologous highly conserved amino acids. The rates of consistency and similarity were 26% and 47% between *PbCPR* and *PvCPR*. The tertiary structure of *PbCPR* showed forceps with wings, but no wings in the tertiary structure of *MmCPR* (Figure 5).



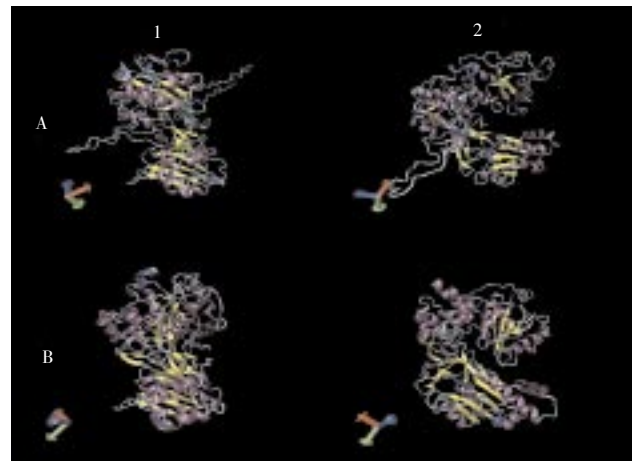
**Figure 3.** Secondary structure of *PbCPR*.

Red alphabet represent conserved amino acid residue,  = conserved domain, H =  $\alpha$  helices, E =  $\beta$  strands, e = exposed, N = NORs,  = N-glycosylation site,  = cAMP- and cGMP-dependent protein kinase phosphorylation site,  and  = Protein kinase C phosphorylation site,  = Casein kinase II phosphorylation site,  = Tyrosine kinase phosphorylation site,  = N-myristoylation site.



**Figure 4.** Tertiary structure of *PbCPR*.

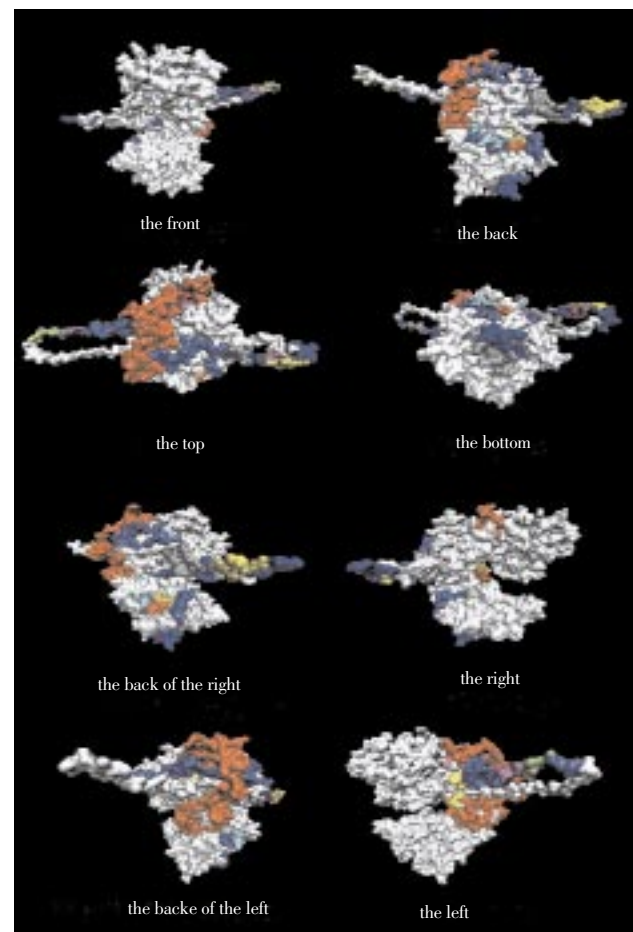
Red = NORs, tan = FMN–red superfamily, dehre = FNR–like superfamily, blue = non domain, pink = N-glycosylation site, grass green = cAMP- and cGMP-dependent protein kinase phosphorylation site, yellow = Protein kinase C phosphorylation site, black green = Casein kinase II phosphorylation site, gray = Tyrosine kinase phosphorylation site, cyan = N-myristoylation site.



**Figure 5.** Comparison between the structure of *PbCPR* and *MmCPR*. A and B represent *PbCPR* and *MmCPR*, respectively 1 and 2 represent the front and the side, respectively.

### 3.6. Unique sites of *PbCPR*

137aa–200aa, 201aa–218aa, 220aa–230aa, 232aa–248 aa, 269aa–323aa, 478aa–501aa and 592aa–606aa of *PbCPR* were expose on the surface of the protein, and had no homology to *MmCPR*. 137aa–200aa, 201aa–218aa and 220aa–230aa were located in NORs. 232aa–248aa and 269aa–323aa were in front of the left winglike structure of the protein, 478aa–501aa was in the right, 592aa–606 aa was in the undersurface (Figure 3 and 6).



**Figure 6.** Unique sites of *PbCPR*.



White = highly similar with other species; other colors = the unique sites of *PbCPR*; red = NORs, blue = non functional sites; purple = N-glycosylation site; yellow = Protein kinase C phosphorylation site; green = Casein kinase II phosphorylation site; grey = Tyrosine kinase phosphorylation site; cyan = N-myristoylation site.

#### 4. Discussion

NO widely exists in organisms. It is an important signal molecule and the key factor in the anti-malarial mechanism[2]. NOS is an important enzyme in NO synthesis process, and mainly provide the NO which participates in inhabiting or killing *Plasmodium*[3,4]. NOS can be found in both normal red blood cell (RBC)[5] and *Plasmodium* infected RBC[6]. Recently, NO has been found in *Plasmodium*. NOS of *Pb* and other *Plasmodium* species are not available in bioinformatics retrieval, whereas CPR partly similar to *HsNOS* and *MmNOS* can be gained. Therefore, *Plasmodium* including *Pb* may have no NOS gene, and NO in *Plasmodium* may originate mainly from other sources.

Both CPR and NOS belong to the family of diflavin reductases[7]. The main function of CPR are transporting electron and taking part in drug metabolism. CPR provides electron transport to cytochrome P450 monooxygenase system, cytochrome C, cytochrome b5, heme oxidase, squalen monooxygenase and 7-dehydrocholesterole reductase. It can catalyze reductive metabolism of prodrugs[8], and produce reactive oxygen species (ROS) by oxidation of NADPH[9]. Gene mutation of CPR causes Antley-Bixler syndrome (ABS) that is an autosomal recessive hereditary disease, developmental disability, disorders of drug metabolism, etc[10–13].

It is predicted by bioinformatics analysis that different from *MmCPR* which is in cytoplasm, *PbCPR* is located in cell nucleus, and has 14% sequence locating in NORs forming section. It is still possible that the subcellular localization of *PbCPR* may be one immune evasion mechanism.

Amino acids sequence alignment and evolution analysis indicates that *PbCPR* has closest relationship with *PvCPR*, but distant from *PfCPR*. Therefore, the study on *Plasmodium berghei* may be more suitable to *Plasmodium vivax* than *Plasmodium falciparum*.

It is still unclear now what kind of role CPR of *Plasmodium* species play in the growth and reproduce of *Plasmodium* species, and how CPR gene mutation affect the growth and reproduce of *Plasmodium* species. Considering the role of CPR in electron transporting and drugs metabolism, it can't rule out the possibility that CPR of *Plasmodium* species may be target for potential anti-malaria drug and vaccine, and the possible resistance sites of anti-malaria drug. According to amino acids sequence, subcellular localization of *PbCPR* and *MmCPR*, the function of CPR and these diseases due to CPR gene mutation in mammals, it can be concluded that *PbCPR* is a possible target for drugs and vaccines, with 137aa–200aa, 201aa–218aa, 220aa–230aa, 232aa–248, 269aa–323aa, 478aa–501 aa and 592aa–606aa being the ideal target domains.

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#### Conflict of interest statement

We declare that we have no conflict of interest.

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